Supporting information for FAIMS and native mass spectrometry: Analysis of intact protein assemblies and protein complexes.

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Supporting Information

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Experimental details

Materials

Protein standards and the detergent C8E4 were obtained from Sigma-Aldrich (Dorset, UK). Water and methanol (both LC-MS grade) were purchased from Fisher Scientific (Loughborough, UK). HPLC-grade ammonium acetate was purchased from J. T. Baker (Deventer, The Netherlands). Solutions of the protein standards carbonic anhydrase (CAH, 7 μM, 100 mM ammonium acetate) and concanavalin A (ConA, 1 μM, 200 mM ammonium acetate) were prepared and used without further purification. Yeast alcohol dehydrogenase 1 (ADH) was dissolved in 150 mM ammonium acetate to a concentration of 6 μM and buffer exchanged three times into ammonium acetate (150 μM) using size exclusion chromatography spin columns(Micro BioSpin-6, Bio-rad, Hercules, CA). The original buffer was removed from the column by centrifugation at 1000 *g* at approx. 20 °C. Columns were loaded with ammonium acetate solution and centrifuged for 1 minute at 1000 *g* 5 times (500 μL per cycle). Finally, 70 μL protein solution was added and the eluent collected by centrifugation at 1000 *g* for 4 minutes.

Kidney from wild-type mice (extraneous tissue from culled animals) was the kind gift of Dr Caroline Chadwick (University of Birmingham). Tissue was frozen in liquid nitrogen and then stored at −80 °C until sectioned. Kidney tissue from an orally dosed[36] adult male Hans Wistar rat was the kind gift of Dr Richard Goodwin (Astra Zeneca). (The drugs were administered as a cassette containing erlotinib, moxifloxacin, olanzapine, and terfenadine (at 10, 25, 10, and 25 mg/kg, respectively)). The animal was euthanized 6 hours post dose. All tissue dissection was performed by trained AstraZeneca staff (project licence 40/3484, procedure number 10). The kidney was snap frozen and kept at -80 °C until tissue processing.

Thin tissue sections from both mouse and rat kidney were prepared at 10 μm thickness with a CM1810 Cryostat (Leica Microsystems, Wetzlar, Germany), thaw mounted onto glass microscope slides and stored at -80 °C until analysis.

FAIMS-MS

Both direct infusion nanoelectrospray (nESI) and liquid extraction surface analysis (LESA) analyses were performed using a Triversa NanoMate (Advion, Ithaca, NY). Ionization was initiated with a potential of 1.75 – 1.85 kV and a back pressure of 0.2 PSI. Emitters on the ESI chip had an internal diameter of approx. 2.5 μm. Contact LESA was performed by aspirating 5 μL extraction solvent (either 200 mM ammonium acetate + 5% methanol, or 10 mM ammonium acetate + C8E4, as stated in main text) into a conductive pipette tip and moving to the sampling location on the tissue. The

pipette tip was pressed into the tissue surface and 2.5 μL solvent dispensed. After one minute, 3 μL was aspirated and moved to the ESI chip for ionization.

The FAIMS Pro device was mounted to an Orbitrap Eclipse Tribrid mass spectrometer (both Thermo Fisher, San Jose, CA) in front of the high-capacity ion transfer tube. FAIMS was set to the standard resolution mode (both electrodes at 100 °C), with supplementary FAIMS gas flow (N_2 purity approx. 99.5%) of 0.1 L/min. The FAIMS Pro front plate was set at 250 V and the dispersion voltage was -5 kV. Compensation voltage (CV) scans for optimization of transmission of each analyte were performed for the range -60 V to -10 V in steps of 1 V per scan. Each scan comprised 5 microscans each with a maximum injection time of 350 ms. Subsequent static FAIMS analyses were performed, in which the optimum CV (chosen for transmission of charge states observed in the absence of FAIMS) was applied.

The mass spectrometer was tuned and calibrated according to the manufacturer's instructions for high mass analysis using FlexMix standard mixture (Thermo Fisher). The ion transfer tube temperature was set to 250 °C. Data were acquired in the "Intact Protein" mode, with the ion routing multipole pressure set to "Standard pressure" (0.008 Torr) with high purity N_2 (99.998%). Orbitrap resolution (defined at *m/z* 200) and *m/z* range was set per analysis: CAH; 120,000, *m/z* 2000 - 4000, ConA; 30,000, *m/z* 2000 – 8000, ADH; 30,000, *m/z* 2000 – 8000, mouse kidney; 15,000, *m/z* 3000 – 6000, rat kidney; 15,000, *m/z* 3000 – 5000. Automatic gain control (AGC) target was 100% for CAH and 500% for all other analyses with a maximum injection time of 500 ms. A source collision potential of up to 65 V was used to desolvate and desalt protein standards; 3 V and 85 V were used when analyzing mouse and rat kidneys respectively. The static FAIMS mass spectra presented are comprised of between 25 and 126 scans.

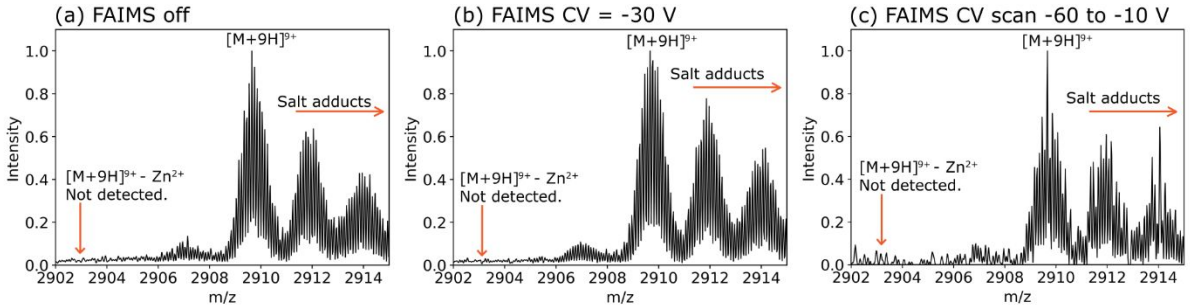


Figure S1: Expanded m/z range showing the 10+ ion of holo-CAH obtained (a) with the FAIMS voltages off; (b) under static FAIMS conditions (CV = -30 V); (c) following a CV sweep from -60 V to -10 V. No evidence for the apo-protein (i.e. without the Zn^{2+} ion) was detected.

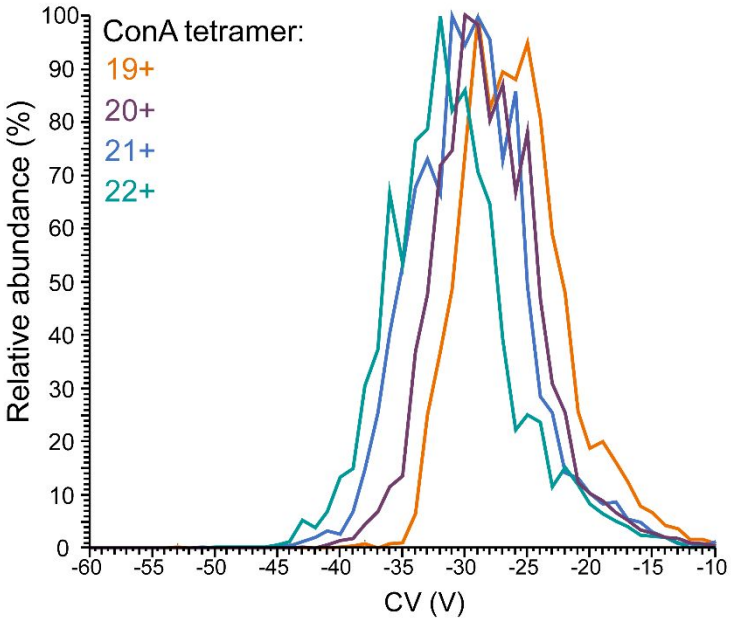


Figure S2: Extracted ion chromatograms for ConA tetramers of four different charge states.

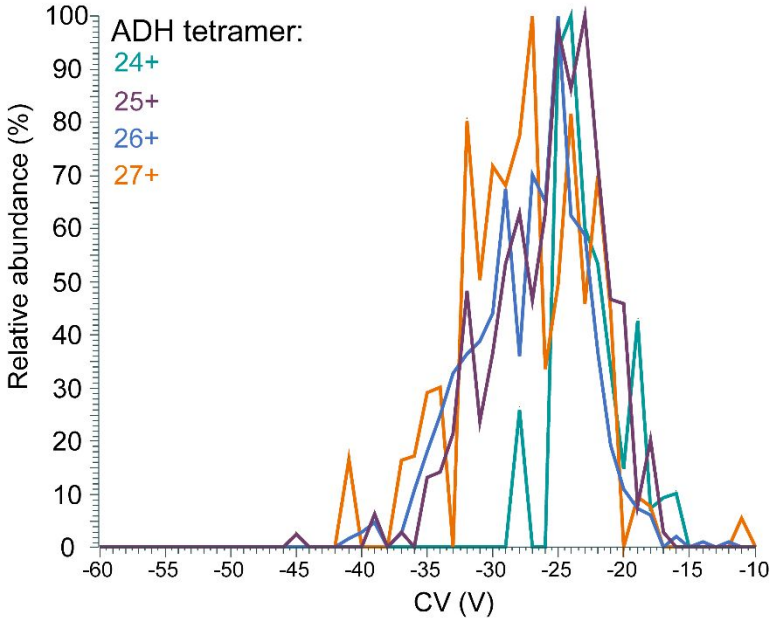


Figure S3: Extracted ion chromatograms of ADH tetramers of four different charge states.

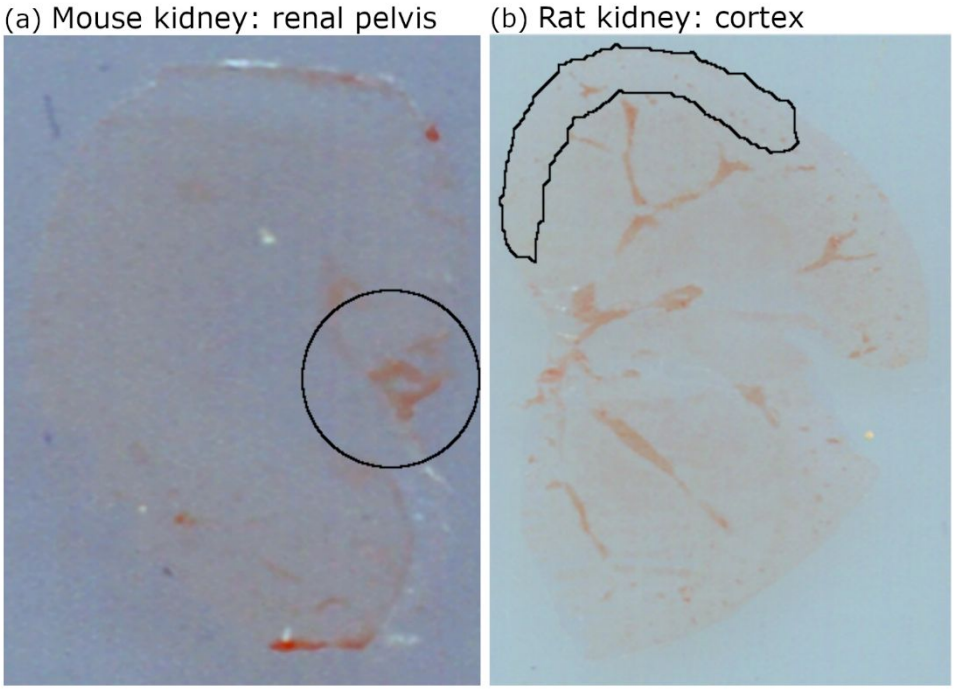
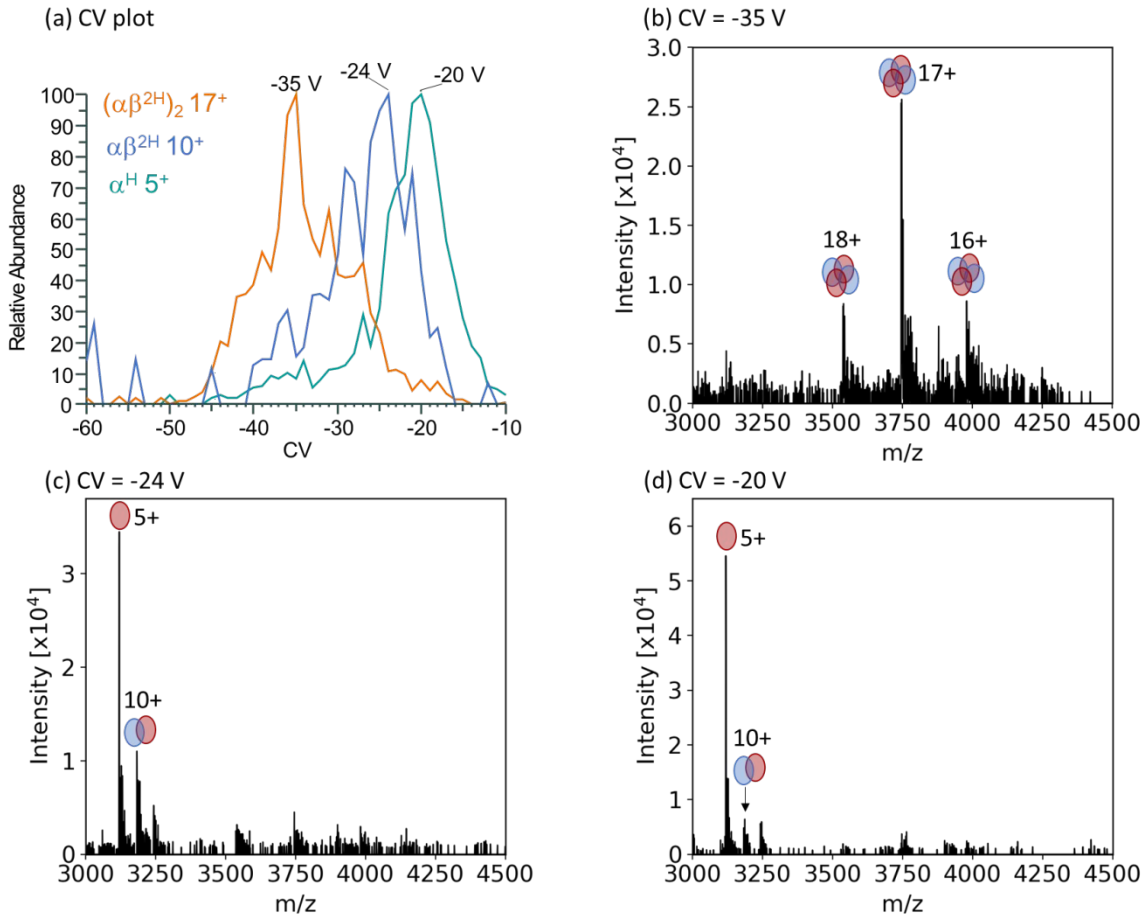


Figure S4: Photograph of (a) mouse kidney section with renal pelvis region indicated; (b) rat kidney section with region of the cortex sampled by LESA indicated.



Supporting Figure 5: Optimization of CV for transmission of hemoglobin ions. (a) Extracted ion chromatograms for hemoglobin complexes; (b) single scan FAIMS mass spectrum at CV = -35 V. Heterotetramer ions were predominantly detected; (c) and (d) single scan FAIMS mass spectrum at CV = -24 V and CV = -20 V. Heterodimer (10+) and holo-alpha monomer (5+) ions were detected, with the latter having the greater intensity at -20 V. A CV of -35 V was chosen for static CV analysis of mouse kidney, for best transmission for the heterotetramer ions.

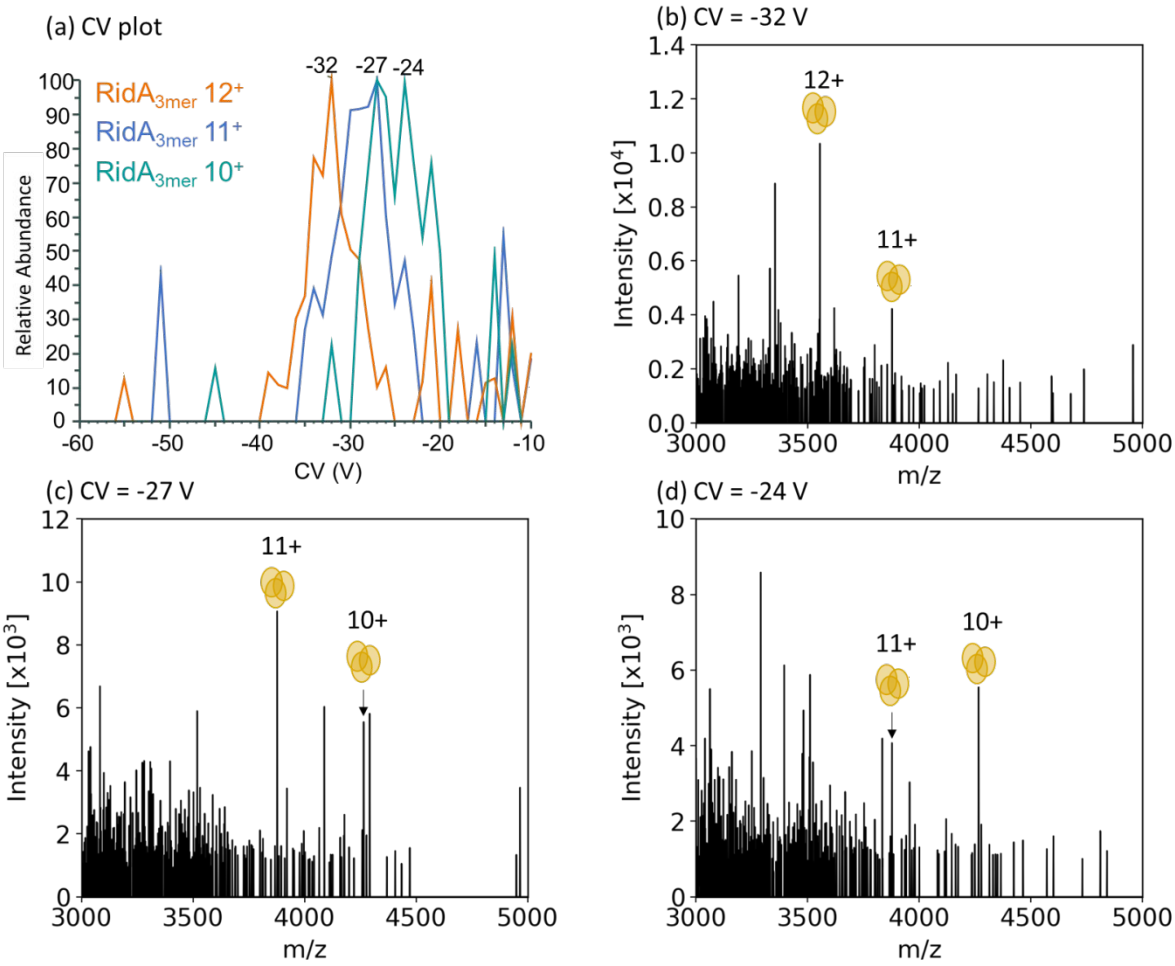


Figure S6: (a) Extracted ion chromatograms for RidA homotrimer ions of different charge states. (b) Single scan mass spectrum at CV = -32 V. The dominant peak corresponds to 12+ ions. (c) and (d) Single scan mass spectra at CV = -27 V and -24 V. Peaks corresponding to 11+ and 10+ ions were dominant. A CV of -29 V was chosen for static CV analysis, to transmit all three RidA charge states.